/ / التاريخ

الموضوع: Pratical Microbiology *

Microbiology: is the science which study microorganism *

Bacteria → Bacteriology
fungi → Mycology
virus → virology
parasite → parasitology

*Lab Safety:

* personal Lab safety:

1] wear Lab coat, gloves, Mask *
2] wash hand before and after work *
3] Don't eat, drink or smoking in Lab *
4] sterilization of bench before and after work *
5] sterilization of equipment *

What are Methods transport of infection?

1] ingestion (ابتلاع) * 2] Injection 3] Inhalation *

* sterilization: killing of spore forming and non spore forming of microorganisms *

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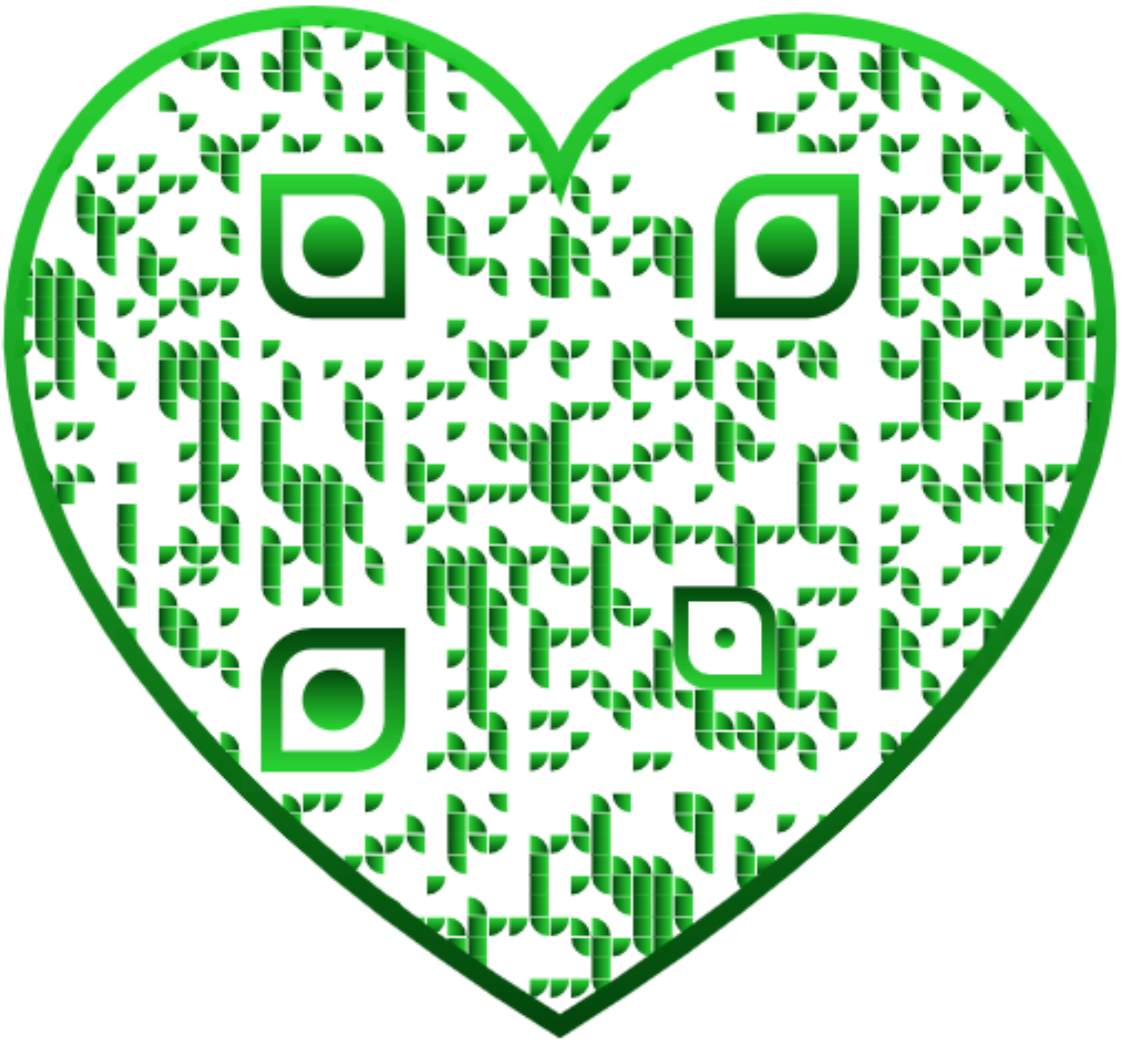

الموضوع : _____ التاريخ / /

Disfectant: killing only non spore forming

Type of sterilization:

physical

1] Heat → Dry → Flaming
→ Incineration
→ Hot air oven
160-180°C for 1-2 hr

→ moist → Boiling (at 100°C for at last 30 min)
→ Auto clave ((121°C, for 15-20 min)) 1 bar

2] Radiation (UV, X-ray)

3] Filteration «fluid»

chemical

e.x: alcohol, Iodine

Type of samples: «culture»

1] upper respiratory Tract infection

-Mouth- tonsils - throat - Nasal - phyrnax ect.

Collect by cotton swab

2] lower respiratory Tract infection:

Lung - sputum - pleural fluid - (steroid container)

3] GIT «stool, rectal swab»

4] UIT «urine, Mid stream urine»

5] menigitis «C-S-F» cerbro spinal fluid»

الموضوع : _____ التاريخ / /

6] Bacteraemia , Sepsis (تعفن الدم) → Blood or Bone marrow*

7] eye, ear swab

8] Pus , effusion (انصباب) , Abscess*

Micro-organism (كائنات حية دقيقة)

equipment in Microbiology Lab:

1] Autoclave : used for sterilization of culture Media, solution and equipment before used at 121°C for 15-20min*

2] hot air oven : used for sterilization of pipettes, glass — at 160-180°C for 1-2hrs

3] petri-dish : for storage of culture for cultivation of bacteria*

4] wire loop or plastic loop : used for Routin inoculation of agar slopes and making streakplate*

5] cotton swab : for collection of swab samples as eye, ear, Nasal, Mouth, throat ..... ect swab*

6] flask, Beaker, cylinders : used in preparation of culture media*

7] filter paper : for filteration fluids*

8] paster pipettes : Transfer measured volume of solution

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التاريخ / /

الموضوع :

[9] Microscope slides: used for microscopic observation*

[10] Cover slips: used for microscopic observation*

[11] incubator: used for growing bacteria culture*

[12] Centrifuges: used for centrifuge sediments particles cells, bacteria ....ect)

[13] Normal salin 0.85% NaCl solution: used for making suspension bacteria

[14] Distilled water: for preparation of culture media

[15] water-bath: suitable temperature for keeping Melted Media and used for seralogical test.

[16] Test tube rack: to hold tubes*

[17] balance: used for weight culture media powder and chemicals.

[18] Bunsen burner: used for sterilization of wire loop and metal forceps*

التاريخ / / الموضوع: _____

Lab diagnosis of bacteria

Sample « swabs, urine, CSF .....)

Direct examination

stains → Bacteria

→ pus cell (W.B.C) Neutrophile

thin blue granules

3-5 lobes

Culture

بعين نعمل زراعة

Type of Bacteria stains

Simple

one stain

e.x: Methylene blue *

- Safranine

size, shape, arrangement)

Differentiation

more than one stain *

- Gram stain

Gram +ve Gram -ve

- Ziehl Neelsen stain (AFB) acid fast

- Albert's stain « diphtheria

Gram stain « 1) size, 2) shape

Cocci

Bacilli

* arrangement → Claster

→ Chain

→ diplococci

/ / التاريخ

الموضوع :

Classified the bacteria

- Gram +ve
  - purple
  - Cell wall large amount of peptidoglycan
    - Cocci
    - Bacilli
- Gram -ve
  - pink, red
  - cell wall low amount of peptidoglycan
    - Cocci
    - Bacilli

* Contents of Gram stain:

1] Basic stain (crystal violet) → purple
2] Mordant stain (Iodine)
3] decolorization (alcohol
4] Counter stain (safranine) → Red

Materiles:

- sample or (culture)
- glass slide, wireloop, D.W, Bunser burner*

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التاريخ / /

الموضوع : _____

Method :-

1] preparation of smear. → one drop of D.W and touches of culture

Dry تثبيت

2] fixation by flamming «3 times »

3] Cover with crystial voliet (1 minute)

4] washing

5] Cover with Iodine (1 minute)

6] washing

7] Cover with decolorization (alcohol) upto 5sec

8] washing

9] Cover with safranine) 1 minute

10] washing → Dry

/ / التاريخ

الموضوع: Stain of bacteria

1] Gram stain → G+ve purple → cocci (كرويه) / bacilli (عصويه)
(تحدد البكتريا اذا كانت G+ve, G-ve)
→ G-ve Red → cocci / bacilli

2] Ziel Nelseen stain → TB (Mycobacterium tuberculosis) (تسبب بكتريا)

3] Albert stain → diphtheria (Corynebacterium diphtheria) (تسبب بكتريا الخناق)

Ziel Nelseen stain

Uses: used to diagnosis of tuberculosis (acid fast bacilli)

Don't stain with Gram stain due to the cell wall contain complex fatty acid*

*Compenent of Ziel Nelssen

1- Carbol fuchsin stain (Basic) (Red)

2- acid alcohol 3% decolorizer

3- Methylene blue (Counter stain) (Blue)

Materiles:-

wire loop, glass slide, Bunzen burner, water (N.S)

Sample (sputum)

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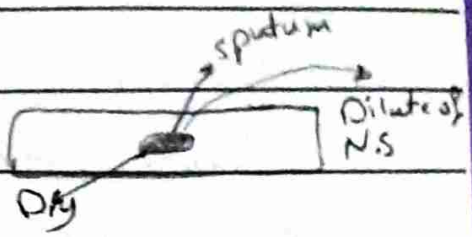
الموضوع : _____

Methodi:-



1] preparation of smear

2] Fixation → by Absulate alcohol

3] Cover with Carbol funchsin stain + heating Until steam Comes out(why)
لحد ما يطلع بخار (ليه؟) عشان تذوب الـ wax اللي هو الـ cell wall عشان الصبغة تدخل

4] Cool, washing

5] Cover with acid alcohol 5min

6] washing

7] Cover with methylene blue 1-2min

8] washing → Dry → examination by microscope

العدسة 40 → 100 · زيت

Report:-

Acid Fast bacilli :- red → AFB. are seen

Background → blue → AFB not seen

Albert stain

used to diagnosis of diphtheria (Corynebacterium diphtheriae)

G+ve bacilli → Gram stain

Rod → purple

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التاريخ / /

الموضوع :

Albert stain «green»

Metachromatic granules → Brown
→ green

energy storage of bacteria

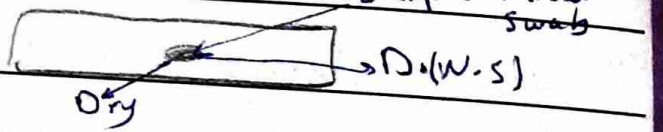
اذا شفنا Brown بتلون بكتيريا

* Compented of stain

- R_1 « Malachite green)
- R_2 « Iodine»

Method :—

1] Preparation of smear



2] fixation

3] cover with malachite green 3-5 min

4] washing

5] cover with Iodine 1min

6] washing → Dry → examination

Report:

Metachromatic granules are seen اللون البني

→ Corynebacterium diphtheria *

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الموضوع : _____

Culture Media:

⇒ artificially Media contain (protein, sugar, Vitamine, Minerals, water) to growth bacteria *

* Forms of Media:-

1] Solid ⟶ 2.5% agar مادة هلامية

2] semi solid ⟶ 0.5% agar

3] fluid ⟶ 0 No agar

* Types of Media:

1] Basic Media (pale yellow) solid

A] Neutrient agar

B] Mueller hinton agar

Uses: to growth all bacteria excepted:

1 Fastidious bacteria

I* H. influenzea (G-ve bacilli) all bacteria

II* Neisseria sp (G-ve cocci) واحدة سبحية

III* Streptococcus pneumonia (G+ve cocci) staphylococci

2] Antibiotic sensitivity test used

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الموضوع :

2] Enriched Media → Blood agar (red)
↳ Chocolate agar (Brown)

Basic Media
+
blood · 5-10% Blood

I] Blood agar 5-10% (blood) solid, red

uses :- to determine the type of haemolysis :-

- β haemolysis :- Clear zone arround the colonies
- α haemolysis :- green zone arround the colonies
- ɣ haemolysis :- No haemolysis

II] Chocolate agar :- بسبب تكسير الـ RBC

5-10% blood → Brown, solid

uses :- to growth of fastidious bacteria

3] selective and differential media

Indicator :- change color of colonies

G-ve bacilli

Lactose Fermenter (L.F) Yellow

Lactose non fermenter (L.N.F) Blue

1] Neutrial red
2] Phenol red
3] Bromothymol blue

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1| Macconkey agar (G-ve bacilli), pink, solid).

Indicator :- Neutrial red

Uses :- different between L.F and L.N.F

colonies : pink yellow

2| XLD agar (stool sample) pink

Indicator :- phenol red

Uses :- different between L.F and L.N.F (*) the same use

colonies yellow pink*

3| SS agar (stool culture) pink

indicator :- neutrial red

uses :- different between L.F and L.N.F / used for (*) Salmonella & Shigella growth

colonies pink yellow

4| CLED agar (used for Urine, Blud)

Indicator :- Bromothymol blue or and red indicator*

Uses :- different between L.F and L.N.F

colonies :- Xellow Blue

5| TCBS « Blue » (*) selective and differentrial media Bromothymol Indicator / used for vibrio cholerae growth*

الموضوع : _____ التاريخ / /

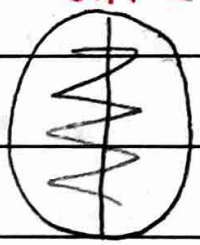
Preparation of Media :-

1] Media powder → 38 gm powder
→ 1000 ml D.W

2] Balance
3] Cylinder
4] Flask
5] Bunzen burner
6] autoclave 121 → 15-20 min
7] Petri-dish

اقتصاص عن طريق اللهب

petri-dish with media, sample, wirloop, Bunzen burner, safety Cabnit



/ / التاريخ الموضوع: _____

Biochemical test

Culture

37C° → 24 hr

growth

Gram stain

G+ve (purple) ∘ strepto ∘ staph

* Biochemical test
* catalase test
* coagulase test

G-ve (red) pink) cocci, Nassiria

* Biochemical test for H2S
* KIA, SIM
citrate, urease test *

soger F
H2S gas

* Biochemical test for G+ve cocci

streptococcus — staphylococcus

1 Catalase test

Use: to differential between staphylococcus (+ve) from streptococcus (-ve)

principle:

Catalase reagent H_2O_2 —Catalase by bacteria→ $H_2O + O_2 \uparrow$ bubbles

Result: staph +ve

Material: reagent H_2O_2, Culture media, slide, Loop

Method: 1 drops of H_2O_2 + touch of colonies by loop *

/ / التاريخ

الموضوع :

result:- Active bubbles within 10sec → +ve (staphylococcus)

No ~ ~ ~ ~ → -ve (streptococcus)

Catalase(+ve) staphylococcus :-

Coagulase test → +ve → staphylococcus aureus

Coagulase test → -ve → staphylococcus epidermidis

Coagulase test → -ve → staphylococcus saprophyticus

use:- to differentiation between staph aureus (+ve) from other staph (-ve)

principle:-

fibrogen (EDTA tube) (plasma) $\xrightarrow[\text{by bacteria}]{\text{coagulase}}$ fibrin (clot)

method:-

1. drop of N.S
2. touch of colonies mix
3. Add 1-2 drop of plasma mix

result:-

Agglutination with 10 sec → (+ve) staph. aureus

No ~ ~ ~ → (-ve) other staph +

Biochemical test for G-ve bacilli

1. KIA (Kligler Iron agar)

التاريخ / /

الموضوع : _____

37°C-24hr [1] sugar Fermentation

Indicator: phenol red

orange

acidic
Fermentation
yellow

alkaline
No Fermentation
pink

[2] Gas production

[3] H_2S ~

[1] Sugar Fermentation (glucose, Lactose)

Yellow
Yellow
Yellow

A/A. glucose & Lactose Fermentation +
- No H_2S production
gas production (+ve)
H_2S black

red
Yellow

: K/A glucose fermentation Lactose non fermentation

red
red

K/K glucose and Lactose non fermentation

[2] Gas production

* bubbles فقاعات
تكسرات في الميديا
* cracks

الـ media تتحرك لفوق
* push up

[3] H_2S production

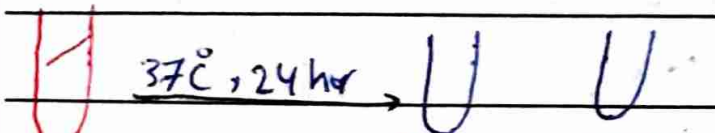
* Black color ⟶ +ve
* No Black ~ ⟶ -ve

/ / التاريخ

الموضوع:

Indicator

2 Citrate test (Bromothymol blue)



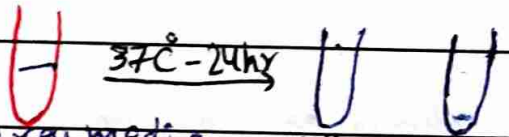
37°C, 24 hr →

before culture green color

blue +ve

green -ve

3 Urease test



37°C - 24hr

urea media pale orange

yellow -ve

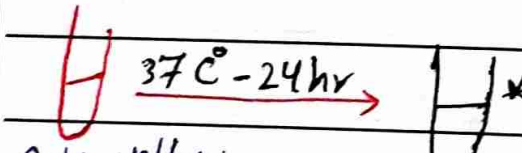
Red +ve

Indicator → phenol red

urea —urease by bacteria→ $NH_3 + Co_2$

alkaline red

4 SIM (Sulfat Indol motility)



37°C - 24hr →

Pale yellow SIM

* H_2S → black +ve

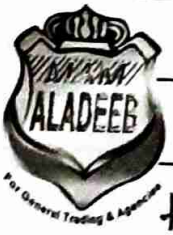
No black -ve

Colorless * Endol drop

red color +ve

no change -ve

* motility

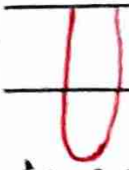


turbidity motile ←

→ growth around the stoping non motile

التاريخ / /

الموضوع : _____



y / y
H_2S = -ve
KIA Gas = +ve

H_2S = -ve
Motility = motile
SIM Indole = +ve

green = -ve
Citrate

yellow = -ve
Urea

E.coli

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/ / التاريخ _____ الموضوع:

Antibiotic sensitivity test

disc diffusion method:-

* Kirby Bauer disc diffusion:-
used by most laboratories routin test

material:-

1* Basic media « Mueller hinton agar → Bunzen burner
2* Culture media → staph.aureus *
3* Anti biotic disc → D.W
4* swab → petri-dish
5* N.S → loop
6* Bunzen burner

methods:-

1] preparation of media 250ml 38gm → 1000, x → 250ml

$\frac{38 \times 250}{1000} = 9.5g \rightarrow 250ml$

2] Suspension (N.S with bacteria)
take 3-5 colonies → 1ml N/S

3] Inoculation the plate

4] add of antibiotic test 6-8 disc

5] incubation at 37 C° for 24hr

* الذي فاعلي sensitive
* الذي مش فاعلي resistant

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التاريخ / / الموضوع :

Mycology

mycology :- is the study of fungi * Eukaryo

mycosis :- is the disease by fungi * infection

structure :-

1] yeast (Graim positive cocci large)

*uncellular. microscopic مجهري

*non filmentous

*fast growth 37C for 24 hr

Culture (large creumy colonies)

2- Mold

* multicellular

*filmentous

* slow growth → R.T — 1-4 weeks

hyphae microscope تحت المجهر

septated مقسم hyphae

Aseptated بدون تقسيم

culture

3] - Dimorphic → 37 → yeast tissue

R.T → mold soil

25 - 30C°

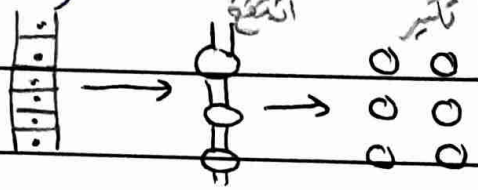
/ / التاريخ _____ الموضوع :

Reproduction of fungi

Asexual → spore formation — sexual.

1] Arthrospore

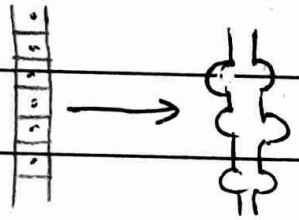
fragmentation of septated hyphae



mold

2] Chlamydo spore

thick wall within hyphae



mold

3- Blastospore → Budding

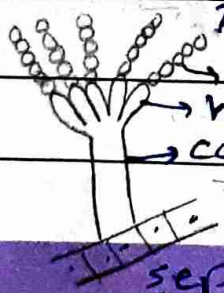
yeast

○ ○ → (budding cell / Blastospore)

4] Conidiospores

upright of stalk without closed sac

A] Pencillum sp



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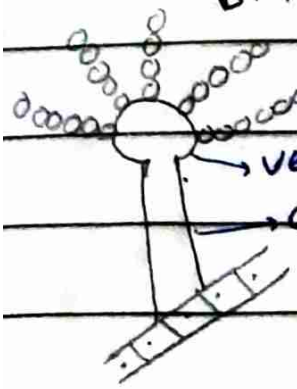
الموضوع :

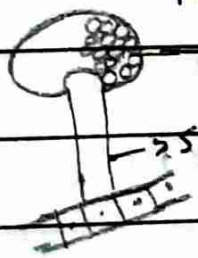
B1 Aspergillus sp



5] sporangio spore

upright of stalk with closed sac

Rhizophus sp



Sample, skin, nail, hair" ⇒ collection of sample

1] skin → Ring worm (داء الحلقة) → fungi edges

↓

scraping of the edges of lesion by scalpel

2] nail → clipping by sterile tweezer

3] hair → plucked out of broken hair

mycology

↓

Labodignosis → culture

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التاريخ / /

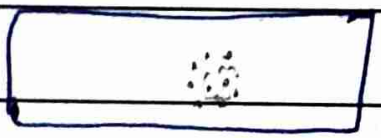
الموضوع :

Direct examination

10% KOH
20% Keratin → dissolve of keratin arround fungi

Keratin في الجلد skin
hair
nail

20gm KOH (powder) + 100ml D.W



glass slide
- add part of sample
- Add 1-2 drop 20% KOH
- cover slip
- incubation → R.T

hair → 5-10 min
Skin → 10-20 min
nail → 30-1 hr

thinck لان الظفر اسمك

Report :- الفطريات 10-40 العدسه 100 البكتريا

Mold
hyphae are seen
yeast are seen
hair
normal
ectothrix out side
endothrix inside
No fungal element are seen (Normal)

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الموضوع: Virology

virus:- one the obligat intracellular.

size:- 20-300nm

Genom → Contain DNA or RNA

Lab diagnosis:-

1] electron microscopic → structure of virus.

2] serological test → Ab → against virus → produced by Immune system.

→ Ag → virus.

3] nucleic acid (DNA, RNA) detection (PCR) most common

4] Culture → cell culture
→ Animals
→ egg culture

Serological test:-

Ag and Ab reaction

1] Immunochromatographic test

2] ELISA test

cast

strep.

Immunochromatographic test

Ag-Ab Complex

Color Line

التاريخ / /

الموضوع :

HBsAg, antibodies → هي الانتيبودي

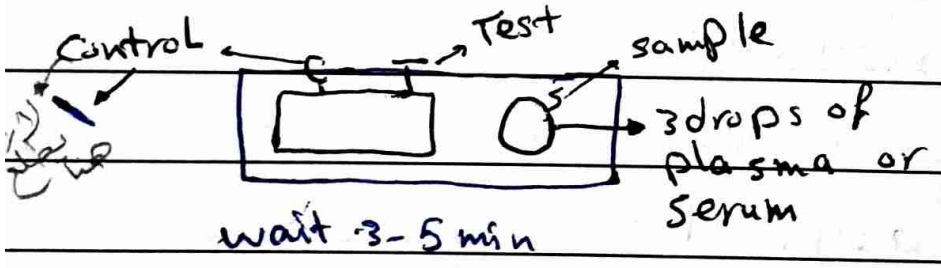
HCV Ab
HIV Ab → antigen → الانتيجين الفيروس

Cassette test :- اسم التيست

principle :-

Ag + Ab ⟶ Ag-Ab complex

Ag: sample serum plasma whole blood
Ab: fixed in cassette
Ag-Ab complex: color line



results :-

two line (C, T) → positive

One line → C → negative

no line
one line-T → invalid

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